

Attorney Docket No.: T5530.CIP (UT-0006)
Inventors: Rao et al.
Serial No.: 09/109,858
Filing Date: July 2, 1998
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the claims of the May 2, 2002 response.

A Request for Continued Prosecution and a Petition to Revive for Unintentional Abandonment as well as the requisite fees are provided herewith. Please enter the following amendments and remarks into the record.

In the Claims:

Please amend the claims as follows:

12. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

- (a) isolating a population of rodent or human multipotent CNS stem cells which generate both neurons and glia;
- (b) incubating the multipotent CNS stem cells in NEP medium;
- (c) replating the multipotent CNS stem cells on laminin in NEP medium in the absence of chick embryo extract to induce cell differentiation;
- (d) removing A2B5+ cells from the differentiating cells via specific antibody capture with an antibody that specifically recognizes A2B5;
- (e) purifying from the supernatant following step (d) a subpopulation of cells expressing embryonic neural cell adhesion

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molecules via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, wherein said procedure uses an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule (NCAM); and

F1 (f) incubating the purified subpopulation of cells in a FGF-containing medium configured for supporting adherent growth thereof to obtain an isolated, purified population of rodent or human CNS neuron-restricted precursor cells, wherein said neuron-restricted precursor cells are capable of differentiating into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

21. (amended) A method of isolating a pure population of rodent or human CNS neuron-restricted precursor cells comprising the steps of:

F2 (a) removing a sample of spinal cord tissue from a rodent or human embryo at a stage of embryonic development after closure of the neural tube;

(b) dissociating cells comprising the sample of spinal

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cord tissue removed from the embryo;

(c) removing A2B5+ cells from the dissociated cells via specific antibody capture with an antibody that specifically recognizes A2B5;

F2 (d) purifying from the supernatant following step (c) a subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic neural cell adhesion molecule antibody that specifically recognizes polysialated neural cell adhesion molecule;

(e) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(f) incubating the plated cells at a temperature and in an atmosphere conducive to growth to obtain an isolated, pure population of neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF for adherent growth, are capable of differentiating into CNS neuronal cells upon replacement of adherent growth supporting medium with retinoic acid containing medium and fail to proliferate or

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F2 differentiate in astrocyte-promoting medium containing FGF and 10% fetal calf serum.

28. (amended) A method of producing postmitotic neurons from a pure population of neuron-restricted precursor cells comprising:

F3 (a) culturing a pure population of neuron-restricted precursor cells which require FGF and are capable of differentiating into CNS neuronal cells but not into CNS glial cells in proliferating conditions; and

(b) changing the culture conditions of the neuron-restricted precursor cells from proliferating conditions to differentiating conditions, thereby causing the neuron-restricted precursor cells to differentiate into postmitotic neurons.

59. (amended) A method of isolating a pure population of mouse or human CNS neuron-restricted precursor cells comprising the steps of:

F4 (a) providing a sample of mouse or human embryonic stem cells;

(b) removing A2B5+ cells from the sample of mouse or human embryonic stem cells via specific antibody capture with an antibody that specifically recognizes A2B5;

(c) purifying from the supernatant from step (b) a

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subpopulation expressing embryonic neural cell adhesion molecule via a procedure selected from the group consisting of specific antibody capture, fluorescence activated cell sorting, and magnetic bead capture, using an embryonic cell adhesion molecule antibody specifically recognizes polysialated neural cell adhesion molecule;

E4 (d) plating the purified subpopulation of cells in feeder-cell-independent culture on a substratum and in a medium configured for supporting adherent growth of the neuron-restricted precursor cells; and

(e) incubating the plated cells at a temperature and in an atmosphere conducive to growth of the neuron-restricted precursor cells, wherein said neuron-restricted precursor cells require FGF and are capable of differentiating into CNS neuronal cells but not into CNS glial cells.

REMARKS

Claims 12, 15, 16, 21, 24, 26-33 and 59 are pending in the instant application. Claims 12, 21, 28 and 59 have been amended in accordance with the Examiner's suggestion during the Telephone Interview conducted August 5, 2002 to clarify that the neuron-restricted precursor cells isolated via the claimed methods are